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Rhinovirus Infections and Associated Respiratory Morbidity in Infants: A Prospective Cohort Study

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ABSTRACT (249/250)

Background. Risk factors promoting rhinovirus (RV) infections are inadequately described in healthy populations, especially infants.

Objectives. To determine the frequency of symptomatic and asymptomatic RV infections and identify possible risk factors from host and environment among otherwise healthy infants.

Methods. In a prospective birth cohort, respiratory health was assessed in 41 term-born infants by weekly telephone interviews during the first year of life, and weekly nasal swabs were collected to determine RV prevalence. In a multilevel logistic regression model, associations between prevalence and respiratory symptoms during RV infections and host/environmental factors were determined.

Results. 27% of nasal swabs in 41 infants tested positive for RVs. Risk factors for RV prevalence were autumn months (OR=1.71, p=0.01, 95% CI 1.13-2.61), outdoor temperatures between 5-10 °C (OR=2.33, p=0.001, 95% CI 1.41-3.86), older siblings (OR=2.60, p=0.001, 95% CI 1.50-4.51) and childcare attendance (OR=1.53, p=0.07, 95% CI 0.96-2.44). 51% of RV-positive samples were asymptomatic. Respiratory symptoms during RV infections were less likely during the first three months of life (OR=0.34, p=0.003, 95% CI 0.17-0.69) and in infants with atopic mothers (OR=0.44, p=0.008, 95% CI 0.24-0.80). Increased tidal volume (OR=1.67, p=0.03, 95% CI 1.04-2.68) and outdoor temperatures between 2-5 °C (OR=2.79, p=0.02, 95% CI 1.17-6.61) were associated with more symptoms.

Conclusions. RVs are highly prevalent during the first year of life, and most infections are asymptomatic. Frequency of RV infections is associated with environmental factors, while respiratory symptoms during RV infections are linked to host determinants like infant age, maternal atopy, or premorbid lung function.

Key Words: respiratory viruses; rhinoviruses; respiratory tract infections; infancy; birth cohort; risk factors.

1. Introduction

Respiratory tract infections can affect lung function and lung growth, especially in infants with developing respiratory and immunologic systems [1,2]. Human rhinoviruses (RVs) are the predominant respiratory tract pathogens during the first year of life, leading to significant short- and long-term burden of disease [3,4]. RV-associated wheeze during early childhood has been linked to later development of asthma, especially in high-risk children with a family history of atopy or genetic predisposition [5-7]. Underlying mechanisms are unclear. RV infections might either predispose infants to the onset of wheeze and asthma, or merely unmask pre-existing susceptibility for later asthma based on other risk factors [8]. On the other hand, asymptomatic RV infections are common and not necessarily associated with subsequent wheezing [7]. A major goal for future research efforts should therefore be to identify host and environmental factors that promote RV illnesses and associated respiratory morbidity in early childhood [5]. To date, most studies investigating RVs have been performed in ill, usually hospitalized, and high-risk infants or in infants from selected populations of a wide age-range [4]. As it is difficult to interpret results drawn from high-risk groups, studies in unselected healthy cohorts with regular surveillance of RV infections in both children with and without respiratory symptoms are required. In this study, we aimed to obtain reliable data on the prevalence of RV respiratory tract infections with and without respiratory symptoms during the first year of life, and the association with host and environmental factors in otherwise healthy infants, enrolled in a prospective birth cohort [9].

2. Materials and Methods

We consecutively studied 41 healthy infants from the ongoing prospective Bern Infant Lung Development (BILD) cohort study (www.birthcohorts.net) [9] between April 2010 and February 2012 during their first year of life. Further information on the study is given in the **Supplemental Digital Content 1, http://links.lww.com/INF/C493**. At the age of 4-5 weeks, we performed lung function measurements and assessed risk factors in pre- and perinatal history by personal interview using standardized questionnaires [9]. During the first year of life, weekly nasal swabs were obtained and study nurses performed weekly standardized telephonic interviews to assess respiratory health (for details see **Figure, Supplemental Digital Content 2**, http://links.lww.com/INF/C494, where week-by-week RV detection and respiratory health status is given for all study infants). Informed consent was obtained from the parents. The study was approved by the Ethics Committee of the Canton of Bern (reference number: 114/11).

We performed diagnostic virology at the Virology Laboratory of the University's Hospital in Geneva. Samples were analyzed by separate qualitative Taqman real time polymerase chain reaction (RT-PCR, One-step-Kit, QIAGEN, Hilden, Nordrhein-Westfalen/Germany) using primers and probes encompassing the UTR region, as previously described [10,11]. This assay detects all known human rhinoviruses, including the recently described rhinovirus C types.

Research nurses weekly assessed the child's respiratory health status during the week the nasal swab was taken by telephonic interview [9]. Using a standardized questionnaire [12,13], lower respiratory symptoms (cough, wheeze, and breathing difficulties) were assessed during daytime and nighttime hours and graded into a symptom score with high sensitivity for lower respiratory tract symptoms (**Table, Supplemental Digital Content 3, http://links.lww.com/INF/C495**) [14]. Rhinitis (runny or blocked nose) was independently

assessed as the most common upper respiratory symptom. For data analysis, we combined upper and lower respiratory symptoms.

Host and environmental factors were assessed by personal interview using standardized questionnaires at 4-5 weeks postnatal age, and at the time of the weekly standardized phone interviews [9,12]. These included time-invariant factors like e.g. sex and time-variant factors like e.g. breastfeeding. Further information on all included risk factors is provided in the **Supplemental Digital Content 1, http://links.lww.com/INF/C493**.

We investigated the association between putative risk factors (exposures) and prevalence of RV infections (first outcome) or respiratory symptoms during RV infections (second outcome) by multilevel logistic regression with a random effect to correct for clustering on the individual level, using STATA 12.0 for Windows (STATA Corporation, College Station, TX/USA). Exposures of interest included all risk factors assessed by personal or telephonic interview. We used univariable regression models to investigate the effect of each exposure variable separately. A multivariable model was fitted including risk factors with $p<0.1$ in the univariable model. Time-variant variables were included on a weekly basis in the regression models. Results are presented as odds ratios (ORs) with a 95% confidence interval (CI) and *p*-values. Ninety-five percent CIs not including 1 were considered statistically significant.

3. Results

We recruited 41 healthy, term-born infants and intended to follow them for one year with a mean (range) follow-up of 42 (7-47) weeks. All children were initially breastfed while only part of them attended nursery care. For distribution of remaining risk factors see **Table, Supplemental Digital Content 4, http://links.lww.com/INF/C496**. A total of 1325 samples were tested for the presence of RVs. From each infant, a median (range) number of 42 (7-47) nasal swabs was collected, beginning with the sample collection at a median (range) age of 5.1 (4.6-7.4) weeks. RVs were found in 363 (27%) samples. 40/41 (98%) infants had at least

one RV-positive sample during the study period with a maximum of 23 (median number of RV-positive samples 10). Overall, 17 RV-positive samples and 49 RV-negative samples were excluded from the final analysis as data on respiratory symptoms was not collected at these time points. 171/346 (49%) of RV-positive samples were accompanied by respiratory symptoms (rhinitis, cough, wheeze, or breathing problems), while 51% (175/346) were not. RVs were detected in 175/911 (19%) of asymptomatic samples and in 171/348 (49%) of symptomatic samples (**Figure 1**). Prevalence of RV infections varied with the season. In univariable analysis, RVs were more frequently detected during spring, summer, and autumn compared to winter, with highest odds ratios in autumn (OR 2.11; 95% Confidence Interval (CI) 1.45-3.07; p<0.001) (**Table 1**). This was due to peaks in March, June, September, October, and November (**Table, Supplemental Digital Content 5, http://links.lww.com/INF/C497 and Figure 2a**). RV infections were most likely at moderate outdoor temperatures, with highest odds ratios between 5 and 10 °C (OR 2.33; 95% CI 1.41-3.86; p=0.001) (**Table 1, Figure 2b**). The seasonal effect was partly mediated by outdoor temperature. After adjustment for both season and temperature, only autumn months remained significantly associated with RV infections (**Tables 1 and** Table, **Supplemental Digital Content 5, http://links.lww.com/INF/C497**). Infants with older siblings were more likely to have RVs compared to singletons with an OR (95% CI) of 2.30 (1.39-3.81, p=0.001) with one and 2.60 (1.50-4.51, p=0.001) with two or more older siblings. Children who attended day care tended to be more likely to have RVs (OR 1.53; 95% CI 0.96-2.44; p=0.07) (**Table 1**). We found no evidence that the remaining risk factors are associated with an increased risk for RV infections (**Table, Supplemental Digital Content 5, http://links.lww.com/INF/C497**).

In the previous section we described factors associated with RV infections irrespective of symptoms, in this part we describe factors associated with respiratory symptoms if RV was present. Information on respiratory symptoms from telephone interviews was missing in n=66, leaving complete datasets for 1259 (95%) episodes. Infants were less likely to be

symptomatic during RV infections in the first three months of life compared to older age (OR, 0.34; 95% CI, 0.17-0.69, p=0.003) (**Table 2, Figure 2c**). Children whose mothers had an atopic disease (asthma, hay fever, or eczema) showed less respiratory symptoms during RV infections (OR, 0.44; 95% CI, 0.24-0.80; p=0.008). Outdoor air temperatures between 2 and 5 °C were associated with more respiratory symptoms (OR 2.79; 95% CI 1.17-6.61; p=0.02, compared to baseline temperatures ≤ 2 °C) (**Table 2**, **Figure 2b**). Higher tidal volumes at lung function testing were associated with more respiratory symptoms during RV infections (OR 1.67; 95% CI 1.04-2.68; p=0.03). All other factors were not significantly associated with respiratory symptoms during RV infections (**Table, Supplemental Digital Content 6, http://links.lww.com/INF/C498**). In sensitivity analysis, using only severe symptoms as outcomes (according to symptom score ≥ 3 [16], **Table, Supplemental Digital Content 3, http://links.lww.com/INF/C495**), comparable results were obtained. Dividing into upper and lower respiratory tract symptoms did not yield different results (data not shown).

4. Discussion

This birth cohort study is one of the first providing longitudinal surveillance of RV infections combined with a prospective monitoring of respiratory symptoms in healthy infants during the first year of life. It shows that RVs are highly prevalent in healthy term-born infants, and most infections are asymptomatic. Results indicate that frequency of RV infections is associated with environmental factors like season, childcare attendance or older siblings, while the occurrence of respiratory symptoms during RV infections is linked to host determinants like infant age, maternal atopy, or premorbid lung function.

Recent studies describe important short- and long-term effects of early viral respiratory infections, especially RVs, on respiratory health of infants [2,17]. However, it is unclear why many RV infections are asymptomatic. In our study, 51% were asymptomatic, and in 19% of asymptomatic periods RVs were detected. This is in line with previous reports showing virus

detection rates of 10-35% in asymptomatic children [3,18-24] with a higher percentage in young infants [7,25], while studies investigating RV prevalence during symptoms of respiratory illness report prevalence up to 73% [4,26]. Information on RV prevalence in healthy infants is scarce, as most studies investigate it during periods of illness [26-29], hospitalizations [30,31] or scheduled visits [18]. In addition, many studies are conducted in older [25,27,32] or high-risk children [3,5,7,18,20], as summarized in a recent review [4]. Apart from technical false positive results, RVs detected in asymptomatic infants in our study cohort may represent (i) incubation periods before the onset of symptoms, (ii) remnants of previous infections after resolution of clinical symptoms [21], or (iii) truly asymptomatic infection. The role of systemic transcriptional immune responses seems important in this context, but is not yet fully understood [33].

We found that RV prevalence was associated with the presence of older siblings and nursery care attendance, both reflecting a high RV exposition. These findings are corroborated by other studies [19,20,34]. In addition, we report a strong association between prevalence of RV infections and particular months of the year: during March, June, and September - November, infants had a higher likelihood for RV infections compared to the rest of the year. This parallels other reports of RV peak prevalence in spring and autumn [3,18,35] and decreased detection rates in winter [36]. The seasonal effect is partly mediated by outdoor temperature, as after adjustment for both season and temperature, only autumn months remained significantly associated with RV infections. This suggests that especially in autumn months additional factors like e.g. crowding effects might play a role [35].

Several characteristics were associated with respiratory symptoms during RV infections. Infants during the first three months of life were less likely to be symptomatic during RV infections, despite no age effect being observed for RV prevalence. This parallels other studies reporting that infants with RV-associated respiratory disease tend to be older [29,37]. Our study included newborns with no previous exposure to RVs, excluding the possibility of

adaptive mechanisms or immune reactions to prior infections, so our results might reflect the protection of young infants by maternal transferred immunity. The negative association between maternal atopic disease and respiratory symptoms during RV infections is a novel finding. Even after adjustment for infant age, this effect remained significant, and was confirmed in sensitivity analysis using only severe symptoms as outcome. We compared smoke or pet exposure, (duration of) nursery care and duration of breastfeeding between the two groups of infants of mothers with and without atopic disease, regarding the possibility that atopic mothers might limit the exposure to factors favoring respiratory infections in their infants. No difference in the exposure was found between the two groups (data not shown), suggesting that there was no effect of "limited exposure". However, the available numbers of children for these comparisons were rather small. Results need to be interpreted carefully, as numbers are small and data from high-risk populations describe maternal atopy as a strong risk factor in infants for virus-associated wheezing and subsequent childhood wheeze or asthma [5,7,19,38]. Studies in healthy infants suggest that an atopic disease of the mother is an important risk factor for severe RV infections [17,29,39]. Immunological studies describe the suppression of regulatory T-cells, Th1-cells and Toll-like-receptors in the cord blood of infants from atopic mothers with known predominant Th2-phenotype [40], possibly leading to a diminished Th1/TLR-dependent inflammatory capacity against respiratory viruses [14].

The association between premorbid lung function and RV-associated symptoms parallels results from other studies supposing that a special breathing pattern, including increased airway resistance, increased tidal volume and a lower respiratory rate is associated with higher respiratory morbidity in infants [17,41,42]. One can speculate that an increased tidal volume might therefore reflect an adaptive change in breathing pattern due to underlying structural pathology. RV is found in up to 65% of asthma exacerbations in children and adults, suggesting that underlying disease might be an important determinant of RV-associated symptoms [17]. If increased tidal volume serves as a proxy for increased

susceptibility to respiratory infections due to possible underlying pulmonary pathology can, however not be disentangled in this study. Study numbers are small and our results will have to be confirmed in larger cohorts with a follow up later in life, to assess possible associations with long term respiratory morbidity.

Outdoor temperatures with highest rates of RV infections (moderate temperatures) and RV-induced respiratory symptoms (cold temperatures), respectively, did not correspond. A recent study also reported that the peak prevalence and peak severity of RV infections did not correspond. While infections were most frequent in spring and fall, viruses were most likely to cause severe illnesses in winter months (December – February) [18]. Temperature- or season-dependent hypotheses focus on low vitamin D production during winter months with decreased immunity [41]. More, the prevalence of many other respiratory pathogens peaks in winter, possibly enhancing the severity of subsequent RV illness [18,35,37]. Whether outdoor temperature is a risk factor per se, or serves as a proxy for specific behavior and/or indoor temperatures cannot be disentangled in our study.

When interpreting the results of our study some aspects need to be considered. A major strength of this study is that data assessment was not restricted to periods of illness, clinical hospitalizations, or scheduled visits. The study provides longitudinal data on RV prevalence regularly and prospectively assessed on a weekly basis irrespective of symptoms. To assess respiratory symptoms in telephone interviews, we used a standardized questionnaire, assuring a high level of data completeness and standardization. The prospective design of our study reduced the risk of recall bias and reverse causation. Despite a total of 1259 paired observations, only 41 infants mostly from small families of higher socioeconomic class were included. This resulted in low numbers of infants exposed to certain risk factors, e.g. only 15% had been exposed to environmental tobacco smoke, or 19.5% of mothers were atopic. Results involving these small subgroups will have to be confirmed in larger cohorts or different populations, respectively. Second, other infectious agents apart from RVs were not

tested, thus we cannot establish RVs as the cause of respiratory symptoms. However, other pathogens occur less frequently [43], and studies investigating the whole spectrum of viruses known to cause infant respiratory tract illnesses describe no other virus detection in up to 73% of RV-positive infants, strongly suggesting that RV is indeed the causative pathogen [29]. The distribution of symptomatic or asymptomatic episodes, however, is not equal between the particular viruses of the respiratory spectrum. We only detected human rhinoviruses, thus potentially increasing the percentage of RV asymptomatic infections in the present study [18]. In our study sequencing of RV species and (sub-) types was not performed, thus no firm conclusions on differences between RV strains can be drawn. In a different sample of the BILD cohort, however sequencing of RV-positive samples was done to determine RV species and types. In this cohort, RV-A and RV-C were almost equally frequent (38% and 39%, respectively), followed by RV-B (12%) with no difference in the seasonal prevalence of the three species. In total, 74 different RV types were identified (30 RV-A, 8 RV-B and 36 RV-C). Regarding the association of RV-positive samples with respiratory symptoms, only slight differences among the three species were found: 53% of RV-A (95% CI 42-63%) and 51% of RV-C (95% CI 40-61%) positive episodes were symptomatic, while only 42% of RV-B (95% CI 25-61%) episodes were associated with respiratory symptoms. The association with specific RV types did not show any recognizable pattern [44]. In previous studies, rhinovirus C and A strains were shown to cause moderate to severe illness compared with milder infection with RV-B, and RV infections were more likely to cause severe illness in winter months [18,41,45,46]. The use of nasal swabs instead of nasal aspirates, washes, or nasopharyngeal swabs may have diminished the recovery of viruses. However, previous studies showed that these methods lead to comparable viral detection rates, even after sending swabs via regular mail [47].

Early life RV infections are ubiquitous and they hold the potential to be important targets for primary and/or secondary asthma prevention efforts. Generally only mildly symptomatic in

healthy infants, severe RV infections in early childhood are associated with an increased risk for later asthma. It`s not clear whether RV infections predispose infants to the onset of asthma, or unmask pre-existing susceptibility for later asthma based on other risk factors. We report RV as a common pathogen in the airways of otherwise healthy infants during the first year of life, and identified risk factors coming from the host or environment, predisposing for RV infections and separating symptomatic from asymptomatic infections. These results may help to further identify vulnerable populations of infants and disentangle the role of RV in asthma inception.

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Figure legends

Figure 1. Flow chart of study infants with distribution of RT-PCR-results and respiratory symptoms (rhinitis, cough, wheeze, or breathing difficulties). Missing information on symptom reports in n=17[a] and n=49[b] leaves complete datasets in n=1259. 348 (171+177) samples were accompanied by respiratory symptoms (28%), and 911 (175+736) were not (72%).

Fig. 2a. Distribution of RV-positive samples (white columns; dashed line represents mean annual average) and RV-positive samples accompanied by respiratory symptoms (grey columns; continuous line represents mean annual average) in % over months of the year.

Fig. 2b. Distribution of RV-positive samples (white columns; dashed line represents mean annual average) and RV-positive samples accompanied by respiratory symptoms (grey columns; continuous line represents mean annual average) in % at different outdoor temperatures.

Fig. 2c. Distribution of RV-positive samples (white columns; dashed line represents mean annual average) and RV-positive samples accompanied by respiratory symptoms (grey columns; continuous line represents mean annual average) in % over age in months.

Fig. Supplemental Digital Content 2. Timeline of 41 study infants showing weekly respiratory health status and presence of rhinovirus in nasal swabs during the first year of life; green boxes indicate respiratory health, red boxes stand for respiratory symptoms (rhinitis, cough, wheeze, and/or breathing difficulties) and symbol (⊙) indicates presence of RV in the particular week; grey boxes represent no symptom report and/or virus sampling; f stands for female and m for male infants.

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Table 1

Unadjusted and adjusted analysis of factors associated with rhinovirus (RV) infections

	Unadjusted model			Adjusted model [a]		
Variable	OR	95% CI	*p*	OR	95% CI	*p*
Season [b]						
Spring	1.61	(1.12-2.33)	0.01	1.21	(0.69-2.09)	0.51
Summer	1.58	(1.09-2.30)	0.02	1.24	(0.65-2.34)	0.51
Autumn	2.11	(1.45-3.07)	<0.001	1.71	(1.13-2.61)	0.01
Siblings						
one older sibling	2.20	(1.36-3.55)	0.001	2.30	(1.39-3.81)	0.001
≥2 older siblings	2.33	(1.39-3.91)	0.001	2.60	(1.50-4.51)	0.001
Nursery care	1.58	(0.98-2.53)	0.06	1.53	(0.96-2.44)	0.07
Outdoor temperature [c], °C						
2.1-5.1	1.37	(0.86-2.17)	0.18	1.36	(0.85-2.16)	0.2
5.2-10	2.81	(1.81-4.36)	<0.001	2.33	(1.41-3.86)	0.001
10.1-14.4	1.41	(0.89-2.23)	0.15	1.27	(0.68-2.36)	0.5
14.5-17.7	1.90	(1.21-2.99)	0.005	1.75	(0.92-3.33)	0.09
17.8-24.7	1.65	(1.05-2.61)	0.03	1.50	(0.75-2.99)	0.25

Data derived from logistic regression analysis corrected for clustering on the individual level, presented as odds ratio (OR) with 95% confidence interval (CI) and *p*-value (*p*).

Reference variables: Winter season, no older siblings, no current nursery care attendance, outdoor temperature ≤ 2 °C.

[a] Adjusted for all variables listed in the table (significant at the univariable analysis at $p<0.10$ and in the final model).

[b] Spring (March 20-June 20), Summer (June 21-September 22), Autumn (September 23-December 21), Winter (December 22-March 19); further details are provided in the **supplemental digital content 3**.

[c] Samples were divided in 6 equally-sized groups: baseline group ≤ 2 °C (n=227), 2.1-5.1 °C (n=218), 5.2-10 °C (n=222), 10.1-14.4 °C (n=225), 14.5-17.7 °C (n=213), 17.8-24.7 °C (n=220).

Table 2

Unadjusted and adjusted analysis of factors associated with respiratory symptoms during rhinovirus (RV) infections

	Unadjusted model			Adjusted model [a]		
Variable	OR	95% CI	*p*	OR	95% CI	*p*
Age group, months						
0-3	0.40	(0.20-0.80)	0.01	0.34	(0.17-0.69)	0.003
4-6	0.87	(0.44-1.73)	0.70	0.76	(0.37-1.53)	0.43
7-9	1.12	(0.56-2.21)	0.75	1.05	(0.53-2.10)	0.88
Maternal atopic disease	0.40	(0.22-0.72)	0.002	0.44	(0.24-0.80)	0.008
Outdoor temperature, °C						
2.1-5.1	2.69	(1.15-6.30)	0.02	2.79	(1.17-6.61)	0.02
5.2-10	1.91	(0.89-4.08)	0.10	1.95	(0.90-4.25)	0.09
10.1-14.4	1.06	(0.45-2.46)	0.90	1.10	(0.46-2.64)	0.83
14.5-17.7	1.25	(0.55-2.82)	0.60	1.19	(0.51-2.76)	0.69
17.8-24.7	1.09	(0.48-2.48)	0.83	0.94	(0.40-2.22)	0.89
Tidal volume, ml	1.79	(1.15-2.80)	0.01	1.67	(1.04-2.68)	0.03

Data derived from logistic regression analysis corrected for clustering on the individual level, presented as odds ratio (OR) with 95% confidence interval (CI) and *p*-value (*p*).

Reference variables: Age group 10-12 months, no maternal atopic disease, outdoor temperature ≤ 2 °C, tidal volume below mean.

[a] Adjusted for all variables listed in the table (significant at the univariable analysis at $p<0.10$ and in the final model).

Figure 1.

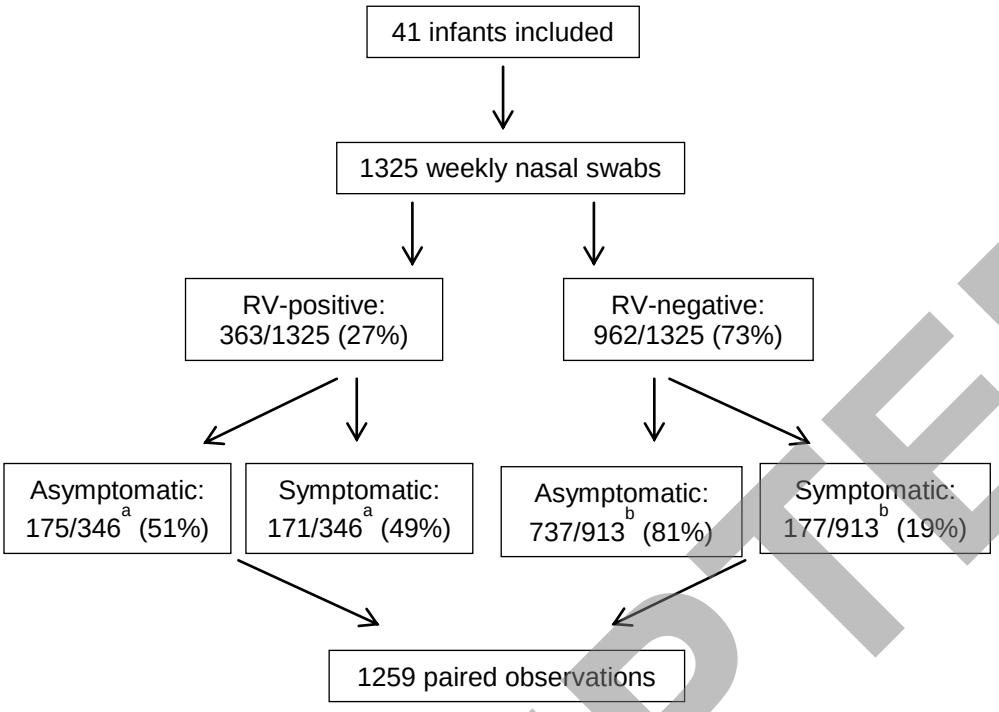
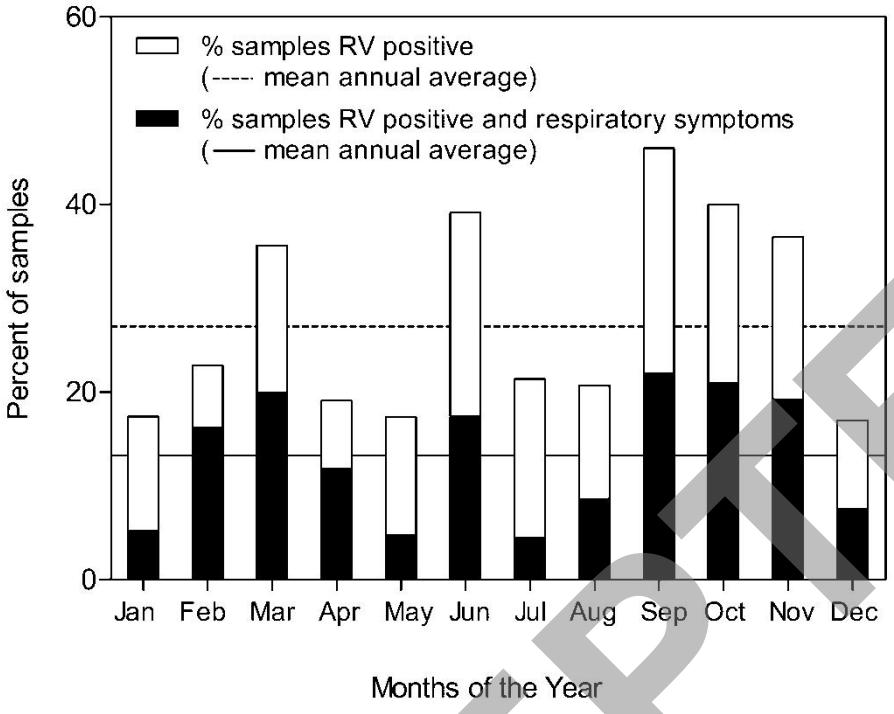


Figure 2.

2A



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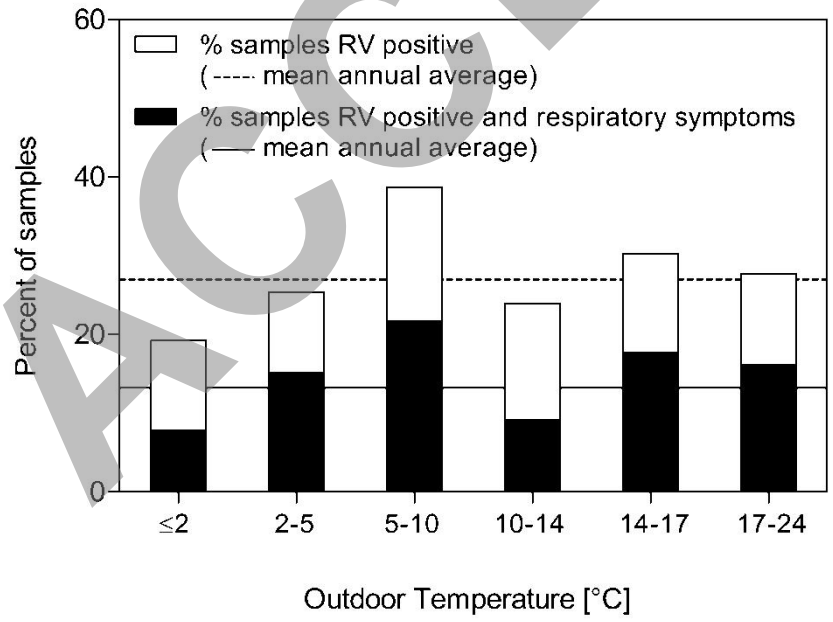
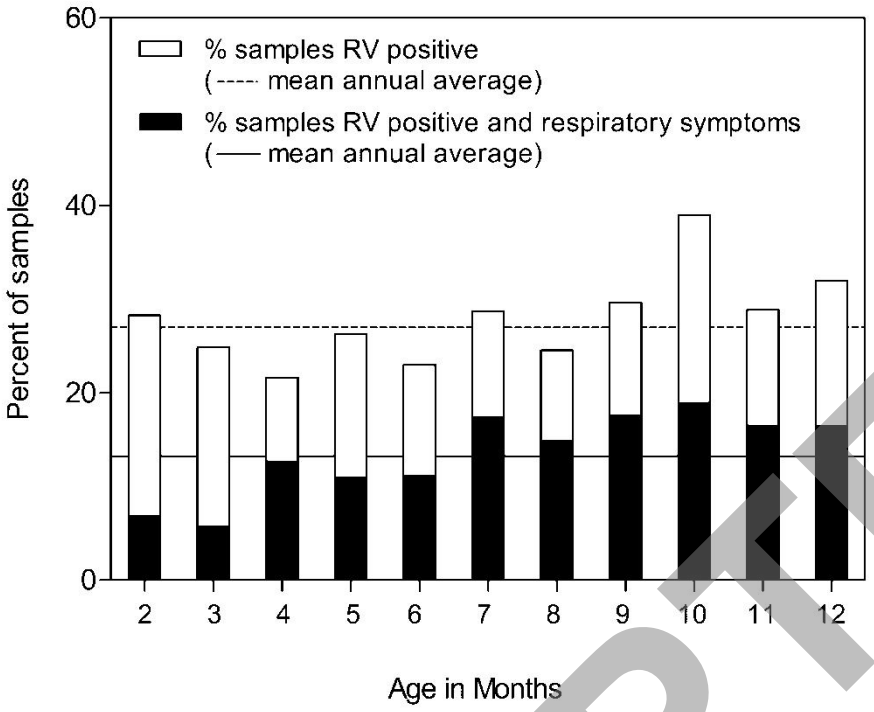


2C

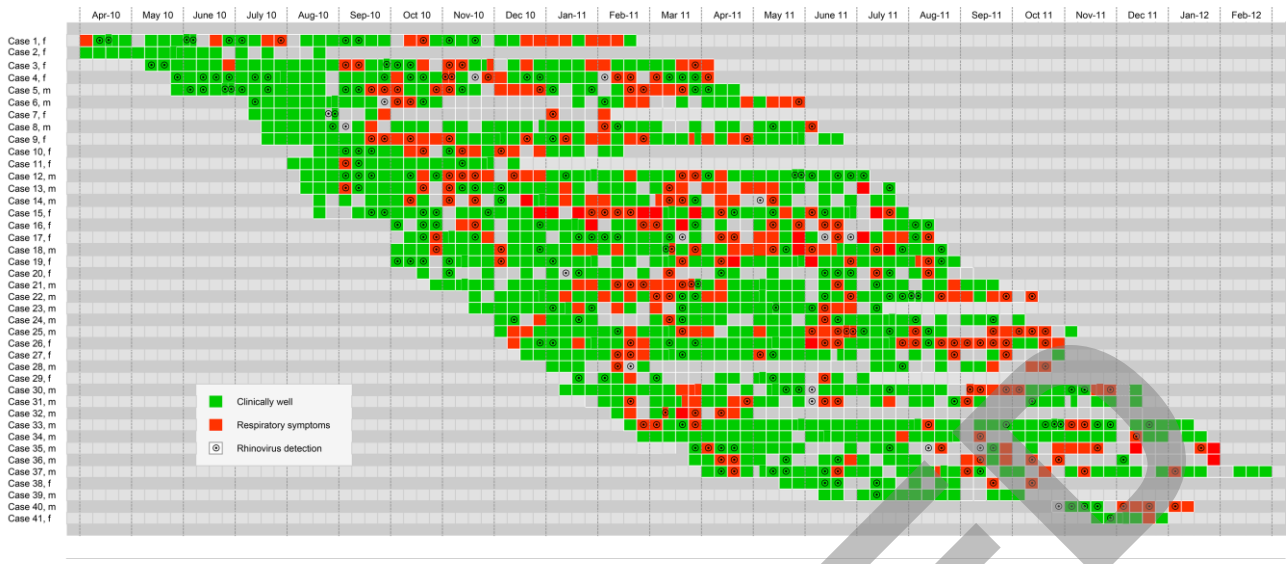


Supplemental digital content 1

Data

For the Bern Infant Lung Development Cohort (BILD) study, pregnant mothers were recruited at maternity hospitals and practices of obstetricians in the agglomeration of Bern through advertisements and interviews. Exclusion criteria for the study were ethnicity other than Caucasian, preterm delivery (< 37 weeks), major birth defects, disease or a later diagnosis of airway malformation or specific chronic respiratory disease [9].

Assessed risk factors included time-invariant factors like sex, maternal atopic disease (maternal asthma, hay fever, or eczema), presence of older siblings, pre- and postnatal smoke or pet exposure, and level of parental education (higher: couples with a higher than average education, i.e. 4 years apprenticeship). We performed lung function measurements including tidal breathing, multiple breath washout and exhaled nitric oxide (eNO) at the age of 4-5 weeks during quiet, un-sedated sleep, as reported previously [15]. Groups were defined based on mean lung function values and categorized into low and high (below/above mean) lung function parameters. Time-variant factors included age, breastfeeding ("current" [yes/no] at time of swab), months of the year, and nursery care ("current" [yes/no] at time of swab). Further, we obtained daily meteorological data assessed during the entire study period from MeteoSwiss, station Bern, e.g. outdoor air temperature (daily mean in °C), and humidity (daily mean in % relative humidity) at the swab sampling day.



Supplemental digital content 2

Figure. Timeline of 41 study infants showing weekly respiratory health status and presence of rhinovirus in nasal swabs during the first year of life; green boxes indicate respiratory health, red boxes stand for respiratory symptoms (rhinitis, cough, wheeze, and/or breathing difficulties) and symbol (⊙) indicates presence of RV in the particular week; grey boxes represent no symptom report and/or virus sampling; f stands for female and m for male infant

Supplemental digital content 3

Table

Symptom score used in weekly phone calls

Symptom score	Daytime symptoms (cough, wheeze [a], or breathing difficulties)	Nighttime symptoms (cough, wheeze [a], or breathing difficulties)
0	None	None
1	Slight; no treatment given	Slight; sleep not disturbed
2	Required treatment but no outside help	Sleep disturbed once; no help required
3	Severe; required help from GP	Sleep disturbed more than once or child needed help
4	Very severe; admitted to hospital	Sleep very disturbed or GP called

[a] A whistling or squeaky noise coming from the chest audible to the parents.

Abbreviations: GP, general practitioner.

Supplemental digital content 4

Table

Characteristics of the study population (N=41 infants with 1325 nasal swab specimens and 1259 symptom observations)

Characteristic		Summary Statistic
Anthropometrics	Boys, n (%)	23 (56.1)
	Gestational age at birth, weeks, (SD)	39.7 (1.1)
	Birth length, cm (SD)	49.7 (2.1)
	Birth weight, kg (SD)	3.4 (0.4)
Measurements	No. of weeks with nasal swabs (SD)	37.4 (9.9)
	No. of weeks with phone interviews (SD)	35.8 (10.0)
	No. of weeks with RV positive swabs (SD)	10.2 (5.0)
	No. of weeks with respiratory symptoms (SD)	10.0 (5.1)
	- Upper respiratory symptoms	8.2 (4.3)
	- Lower respiratory symptoms	4.9 (3.5)
	- Hospitalizations	0
	No. of weeks with RV positive swabs and respiratory symptoms (SD)	4.9 (2.5)
Risk factors	Breastfeeding [a], n (%)	41 (100.0)
	Maternal atopic disease [b], n (%)	8 (19.5)
	Older siblings, n (%)	
	One sibling	21 (51.2)
	≥2 siblings	11 (26.8)

	Nursery care [c], n (%)	10 (24.4)
	Smoke exposure total [d], n (%)	6 (14.6)
	Pet exposure total, n (%)	10 (24.4)
	Parents with higher education, n (%)	31 (75.6)
Lung function	RR, min^{-1} (SD)	45.1 (10.0)
		45.2 (10.5)
	Tidal volume, ml (SD)	33.6 (5.2)
		32.4 (5.5)
	tPTEF/tE, % (SD)	35.7 (9.8)
		34.8 (10.7)
	LCI (SD)	6.8 (0.5)
		6.8 (0.6)
	FRC_{ao}, ml (SD)	106.5 (14.8)
		102.0 (16.0)
	eNO, ppb (SD)	12.5 (6.1)
		14.3 (6.0)
	vNO, $nl \cdot s^{-1}$ (SD)	0.61 (0.31)
		0.63 (0.25)

Data are presented as number (n) and percentage (%) or mean and standard deviation (SD). For lung function parameters *normative values* are given below [15].

Abbreviations: RR, respiratory rate; tPTEF/tE, ratio of time to reach peak tidal expiratory flow to total expiratory time; LCI, lung clearance index; FRC_{ao}, functional residual capacity at airway opening; eNO, exhaled nitric oxide; vNO, NO output, calculated as eNO x expiratory flow for the third quartile of the expiratory cycle [15].

[a] Infants were breastfed for a mean (±SD) duration of 8.2 (2.6) months.

[b] Asthma (n=1), hay fever (n=3), or eczema (n=6).

[c] Infants attended nursery care for a mean (±SD) duration of 7.3 (1.8) months.

[d] Four mothers smoked during pregnancy (1 continuing during first year of life) and two fathers smoked during pregnancy (both continuing during first year of life).

Supplemental digital content 5

Table

Unadjusted and adjusted analysis of (all) factors associated with rhinovirus (RV) infections

	Unadjusted model			Adjusted model [a]		
Variable	OR	95% CI	*p*	OR	95% CI	*p*
Male sex	0.87	(0.59-1.27)	0.461	1.11	(0.75-1.64)	0.61
Age group, months						
0-3	0.76	(0.52-1.12)	0.17	1.19	(0.68-2.09)	0.55
4-6	0.80	(0.54-1.18)	0.26	1.16	(0.67-2.01)	0.59
7-9	1.00	(0.67-1.49)	0.99	1.17	(0.75-1.83)	0.50
Breastfeeding	0.81	(0.60-1.10)	0.18	0.77	(0.49-1.20)	0.25
Maternal atopic disease	1.10	(0.67-1.79)	0.70	0.96	(0.57-1.61)	0.87
Months of the Year						
February	1.54	(0.78-3.03)	0.21	1.60	(0.79-3.26)	0.19
March	**3.01**	**(1.60-5.67)**	**0.001**	1.92	(0.90-4.01)	0.09
April	1.30	(0.65-2.61)	0.46	0.79	(0.27-2.11)	0.63
May	1.14	(0.57-2.24)	0.71	0.74	(0.27-2.02)	0.55
June	**3.49**	**(1.86-6.54)**	**<0.001**	2.24	(0.86-5.83)	0.10
July	1.43	(0.73-2.81)	0.30	0.89	(0.32-2.46)	0.83
August	1.45	(0.74-2.85)	0.28	1.02	(0.37-2.81)	0.98
September	**4.86**	**(2.55-9.26)**	**<0.001**	**3.58**	**(1.41-9.14)**	**0.008**

October	**3.59**	**(1.88-6.85)**	**<0.001**	**2.84**	**(1.30-6.19)**	**0.009**
November	**3.12**	**(1.64-5.95)**	**0.001**	**2.83**	**(1.39-5.77)**	**0.004**
December	1.05	(0.52-2.15)	0.89	1.22	(0.59-2.56)	0.59
Older siblings						
One sibling	**2.20**	**(1.36-3.55)**	**0.001**	**2.08**	**(1.17-3.70)**	**0.01**
≥2 sibling	**2.33**	**(1.39-3.91)**	**0.001**	**3.11**	**(1.70-5.68)**	**<0.001**
Nursery care	1.58	(0.98-2.53)	0.06	1.60	(0.96-2.68)	0.07
Smoke exposure	1.23	(0.75-2.03)	0.42	0.96	(0.61-1.51)	0.86
Pet exposure	0.84	(0.54-1.31)	0.45	0.81	(0.51-1.29)	0.37
Higher education level parents	1.08	(0.54-2.15)	0.84	0.88	(0.50-1.55)	0.65
Outdoor temperature, °C						
2.1-5.1	1.37	(0.86-2.17)	0.18	1.16	(0.71-1.91)	0.55
5.2-10	**2.81**	**(1.81-4.36)**	**<0.001**	**2.00**	**(1.14-3.52)**	**0.02**
10.1-14.4	1.41	(0.89-2.23)	0.15	1.21	(0.57-2.58)	0.62
14.5-17.7	**1.90**	**(1.21-2.99)**	**0.005**	1.50	(0.65-3.44)	0.34
17.8-24.7	**1.65**	**(1.05-2.61)**	**0.03**	1.49	(0.62-3.56)	0.38
Outdoor humidity, rel %	1.01	(0.99-1.02)	0.33	0.99	(0.97-1.01)	0.42
RR, min^{-1}	1.22	(0.84-1.78)	0.30	**1.61**	**(1.02-2.53)**	**0.04**
Tidal volume, ml	1.01	(0.68-1.49)	0.97	1.04	(0.67-1.62)	0.86
tPTEF/tE, %	1.19	(0.82-1.74)	0.36	**1.52**	**(1.04-2.23)**	**0.03**

LCI	1.08	(0.73-1.58)	0.71	0.95	(0.65-1.39)	0.79
FRC_{ao}, $ml \cdot kg^{-1}$	1.16	(0.79-1.70)	0.45	1.19	(0.83-1.71)	0.34
eNO, ppb	**0.64**	**(0.44-0.91)**	**0.02**	0.88	(0.56-1.36)	0.56
vNO, $nl \cdot s^{-1}$	0.73	(0.50-1.07)	0.10	0.79	(0.47-1.33)	0.38

Data derived from logistic regression analysis corrected for clustering on the individual level, presented as odds ratio (OR) with 95% confidence interval (CI) and *p*-value (*p*). Confidence intervals that do not overlap the null value of OR=1 are shown in bold.

Reference variables: Female sex, age group 10-12 months, no current breastfeeding, no maternal atopic disease, January, no older siblings, no current nursery care attendance, no smoke exposure, no pet exposure, education lower than average education (4 years apprenticeship), outdoor temperature ≤ 2 °C, lung function parameters below mean.

[a] Adjusted for all variables listed in the table.

Supplemental digital content 6

Table

Unadjusted and adjusted analysis of (all) factors associated with respiratory symptoms during rhinovirus (RV) infections

	Unadjusted model			Adjusted model [a]		
Variable	OR	95% CI	*p*	OR	95% CI	*p*
Male sex	1.09	(0.66-1.80)	0.74	0.76	(0.36-1.61)	0.48
Age group, months						
0-3	**0.40**	**(0.20-0.81)**	**0.01**	**0.25**	**(0.08-0.77)**	**0.02**
4-6	0.87	(0.44-1.73)	0.70	0.58	(0.19-1.77)	0.34
7-9	1.12	(0.56-2.21)	0.75	1.22	(0.54-2.74)	0.64
Breastfeeding	0.72	(0.43-1.19)	0.20	1.45	(0.61-3.47)	0.40
Maternal atopic disease	**0.40**	**(0.22-0.72)**	**0.002**	0.42	(0.17-1.06)	0.07
Season						
Spring	0.66	(0.34-1.27)	0.22	0.41	(0.13-1.23)	0.11
Summer	0.53	(0.27-1.05)	0.07	0.30	(0.09-1.03)	0.06
Autumn	0.87	(0.45-1.67)	0.67	0.74	(0.31-1.77)	0.49
Older siblings						
One sibling	1.36	(0.66-2.79)	0.40	0.90	(0.30-2.71)	0.86
≥2 siblings	0.89	(0.42-1.91)	0.77	0.59	(0.20-1.74)	0.34
Nursery care	1.29	(0.66-2.53)	0.46	0.83	(0.34-2.01)	0.68

Smoke exposure	1.06	(0.57-1.98)	0.85	0.99	(0.44-2.20)	0.98
Pet exposure	1.00	(0.56-1.79)	0.99	1.42	(0.58-3.47)	0.44
Higher education level parents	0.87	(0.37-2.06)	0.75	1.17	(0.43-3.21)	0.76
Outdoor temperature, °C						
2.1-5.1	**2.69**	**(1.15-6.30)**	**0.02**	**3.47**	**(1.31-9.16)**	**0.01**
5.2-10	1.91	(0.89-4.08)	0.11	2.52	(0.96-6.57)	0.06
10.1-14.4	1.06	(0.45-2.46)	0.92	2.08	(0.63-6.90)	0.23
14.5-17.7	1.25	(0.55-2.82)	0.60	2.24	(0.65-7.77)	0.20
17.8-24.7	1.09	(0.48-2.48)	0.83	1.95	(0.51-7.43)	0.33
Outdoor humidity, rel %	1.01	(0.99-1.03)	0.41	0.99	(0.96-1.03)	0.66
RR, min^{-1}	1.06	(0.65-1.75)	0.81	1.59	(0.70-3.62)	0.27
Tidal volume, ml	**1.79**	**(1.15-2.80)**	**0.01**	2.01	(0.89-4.54)	0.09
tPTEF/tE, %	0.74	(0.45-1.20)	0.22	1.07	(0.56-2.06)	0.83
LCI	0.97	(0.59-1.59)	0.91	1.39	(0.70-2.79)	0.35
FRC_{ao}, $ml \cdot kg^{-1}$	1.07	(0.65-1.75)	0.80	1.04	(0.53-2.03)	0.92
eNO, ppb	1.56	(0.96-2.53)	0.07	1.85	(0.81-4.24)	0.14
vNO, $nl \cdot s^{-1}$	1.11	(0.69-1.82)	0.68	0.62	(0.23-1.71)	0.36

Data derived from logistic regression analysis corrected for clustering on the individual level, presented as odds ratio (OR) with 95% confidence interval (CI) and *p*-value (*p*). Confidence intervals that do not overlap the null value of OR=1 are shown in bold.

Reference variables: Female sex, age group 10-12 months, no current breastfeeding, no maternal atopic disease, winter season, no older siblings, no current nursery care attendance,

no smoke exposure, no pet exposure, education lower than average education (4 years apprenticeship), outdoor temperature ≤ 2 °C, lung function parameters below mean.

[a] Adjusted for all variables listed in the table.